Liposomes containing antigens and excipients such as PBS, saline or phosphate in the interior and cryoprotectant/buffer/(viscosity enhancer) in the exterior phase could also be mixed with the emulsion in the same cryoprotectant combination for the intended application.

3. Lyophilized antigen-bearing liposomes/emulsion combinations

As a stable storage form, this combination is highly preferred because bulk water which could cause chemical instability to lipids and proteins is eliminated. In addition, carbohydrates and amino acids commonly used as cryoprotectants are good support media for microbial growth. The following is illustrative of the design used in lyophilizations to generate dried vaccine formulations.

Antigen-bearing liposomes suspended in suitable cryoprotectant/buffer/(viscosity enhancer) such as those listed in Table 12 are combined with microfluidized emulsions in the same medium and freeze dried at temperatures below the cake collapse temperature for that excipient combination for 24–72 hr. (preferably 24 hr.). In one embodiment, for example, containing sucrose 10% w/v, citrate 10 mM, primary drying is initiated at –40° C. and temperature gradually raised to –10° C. under good vacuum (10–100 microns, preferably 50 microns). After the completion of the primary drying process, the shelf temperature is gradually raised to ambient temperature (10°–20° C. per hour) and left at this temperature and vacuum for an additional 2–10 hours (preferably 4 hours) or until the moisture level in the dried product is reduced to 1–3%. Another variant of this formula consists of drying liposomes and emulsions separately and mixing the reconstituted suspensions to provide the desired formulation. In either case, the lyophilized cakes have acceptable physical appearance and readily reconstitute to provide injectable vaccine formulations.

Lyophilized single- or two-vial formulations by virtue of the low water content have acceptable stabilities for the lipid components and the protein antigens. The formulations can be stored at 2°–8° C. or ambient temperatures.

TABLE 12

Combinations of Cryoprotectants and Viscosity Enhancers used in the Formulations+

Sucrose* (10% w/v)	
Sucrose (6% w/v),	mannitol (1% w/v)
Dextrose (5% w/v)	
Sucrose (9% w/v),	PEG 400, 1450 or 3350 (1% w/v)
Sucrose (9% w/v),	hydrolyzed gelatin (1% w/v)
Sucrose (9% w/v),	gelatin (fish skin, bovine or porcine 1% w/v)
Sucrose (9% w/v),	Povidone 16000 or 40000 (1% w/v)
Glycine,	alanine or proline (0.3 M pH 6–6.5)
Sucrose 9% (w/v)	carboxymethyl cellulose (1% w/v) glycerol (2–20% w/v)

+In addition, phosphate or citrate buffer is used in 10 mM concentration to provide stability to antigens.
*Other sugars, such as lactose, trehalose, maltose, etc., are substituted in appropriate weight amounts to provide equivalent physiologic osmolality.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference at the location where cited.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

1. A vaccine composition, comprising:

(1) an immunostimulating amount of an antigenic substance in association with liposomes, wherein the liposomes have a net negative charge; and

(2) an oil-in-water emulsion comprising a metabolizable oil in a continuous phase surrounding said liposomes, said emulsion being present in an amount sufficient to increase immune responce relative to that of said antigenic substance and liposomes in the absence of said emulsion.

2. The composition of claim **1**, wherein said antigenic substance is also present in said continuous phase in an immunostimulating amount.

3. The composition of claim **1**, wherein said liposomes comprise fusogenic liposomes.

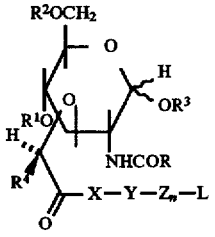
4. The composition of claim **1**, wherein said liposomes and said emulsion are present in a volume ratio of from 1:10 to 10:1.

5. The composition of claim **1**, wherein said liposomes and oil droplets present in said emulsion are present as particles of substantially the same size distribution range.

6. The composition of claim **5**, wherein substantially all of said particles are less than 1 micron in diameter.

7. The composition of claim **1**, wherein said oil-in-water emulsion further contains an immunostimulating amount of a muramyl peptide.

8. The composition of claim **7**, wherein said muramyl peptide is a compound of the formula



wherein R is H or $COCH_3$;

R^1, R^2, and R^3 independently represent H or a lipid moiety;

R^4 is hydrogen or alkyl;

X and Z independently represent an aminoacyl moiety selected from the group consisting of alanyl, valyl, leucyl, isoleucyl, α-aminobutyryl, threonyl, methionyl, cysteinyl, glutamyl, isoglutamyl, glutaminyl, isoglutaminyl, aspartyl, phenylalanyl, tyrosyl, tryptophanyl, lysyl, ornithinyl, arginyl, histidyl, asparinginyl, prolyl, hydroxypropyl, seryl, and glycyl;

n is 0 or 1;

Y is —$NHCHR^5CH_2CH_2CO$—, wherein R^5 represents an optionally esterified or amidated carboxyl group; and

L is OH, NR^6R^7 where R^6 and R^7 independently represent H or a lower alkyl group, or a lipid moiety.

9. The composition of claim **8**, wherein said muramyl peptide is either a muramyl dipeptide or muramyl tripeptide.

10. The composition of claim **9**, wherein said muramyl peptide is selected from the group consisting of muramyl dipeptides and tripeptides linked to a phospholipid moiety through a hydroxyalkylamine moiety.